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Small cytoplasmic RNA associated with polyadenylated RNA is involved in the hormonal regulation of gene expression

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The fraction of small RNA (sacc-RNA) associated with cytoplasmic rat liver poly(A)$^+$ RNA by non-covalent, possibly complementary, interactions has been isolated and studied. Fingerprint analysis and Northern blot hybridization data reveal that the specific changes occur in the population of sacc-RNA in response to glucocorticoid treatment. The close similarity of the oligonucleotide composition of sacc-RNA and RNA-component of small nuclear RNP-acceptor of glucocorticoid hormones has been found. The hypothesis of the involvement of the small RNA in the hormonal regulation of posttranscriptional stages of gene expression in the cytoplasm has been put forward.

Small cytoplasmic RNA; Poly(A)$^+$ RNA; Cortisone induction; Small ribonuclear protein; Posttranscriptional regulation

1. INTRODUCTION

The effects of glucocorticoid hormones involve both the transcriptional and posttranscriptional stages of gene expression [1]. Thus, glucocorticoids have been shown to regulate the nuclear-cytoplasmic transport of mRNA and to stabilize a number of inducible mRNA [1,2]. The molecular mechanisms of the hormone effects upon posttranscriptional events are unknown [1].

In prokaryotes the small natural antisense RNA has been shown to regulate gene expression at posttranscriptional level [3]. In order to investigate the possible role of RNA-RNA interactions in the glucocorticoid posttranscriptional regulation of gene expression in the liver, in this study the fraction of low-molecular-mass RNA associated by non-covalent, possibly complementary, interactions with high-molecular-mass poly(A)$^+$ RNA was isolated and studied.

The fingerprint analysis and Northern blot hybridization have been used on control and hormone-induced small associated RNA populations. The data obtained so far show that small associated cytoplasmic RNA is under hormonal control in liver cells and suggest that the subset of messenger RNA induced by cortisone is associated with a few specific kinds of small RNA. These results allow the proposition that complementary interactions of specific small RNA with the inducible poly(A)$^+$ cytoplasmic RNA are involved in the co-ordinate regulation of posttranscriptional stages of gene expression by the glucocorticoid hormones in the cytoplasm.

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2. MATERIALS AND METHODS

Male Wistar rats (100–120 g) were used. Cortisone-acetate and hydrocortisone in 1 ml of 0.14 M NaCl (5 mg/100 g body wt) were injected intraperitoneally 3 h before the animals were killed. Control rats received 1 ml of 0.14 M NaCl. All animals had been fasting for 24 h.

Nuclear and cytoplasmic RNA was extracted and purified as in [4].

Poly(A)-containing RNA (poly(A)$^+$ RNA) was purified from high-molecular-mass cytoplasmic RNA by oligo(dT) cellulose chromatography [5].

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Isolation of RNase-resistant double-stranded regions from poly(A)$^+$ RNA was performed as in [6].

Electrophoresis of ds-RNA and low-molecular-mass RNA was run on 10% polyacrylamide gel (PAAG), and electrophoresis of poly(A)$^+$ RNA on 2.75% PAAG, and also 0.2% SDS [7].

Low-molecular-mass RNA associated with poly(A)$^+$ RNA was isolated as follows. Purified by two passages through oligo(dT) cellulose, high-molecular-mass poly(A)$^+$ RNA was subjected to denaturation with 90% formamide for 10 min at 65°C or to boiling in water for 10 min. After denaturation, high-molecular-mass RNA was precipitated with 2.5 M NaCl, and non-precipitated low-molecular-mass RNA was collected by precipitation with ethanol. In some cases, after denaturation, poly(A)$^+$ RNA was applied to an oligo(dT) cellulose column, and poly(A)$^-$ low-molecular-mass RNA fraction, which did not bind to the column, was collected. The fraction of low-molecular-mass RNA thus far isolated was called 'small associated' RNA (sacc-RNA).

RNA fingerprint analysis: T_1 RNase fingerprints were prepared according to the method described by Pedersen and Haseltine [8].

Northern blot RNA-RNA hybridization was performed according to Thomas [9]. Poly(A)$^+$ RNA samples were denatured by glyoxal and DMSO (1 M glyoxal/50% DMSO/10 mM Na-phosphate, pH 7.0) and electrophoresed in 1.5% agarose gel in 0.01 M Na-phosphate buffer, pH 7.0. For blotting, GSWP filters, 0.22 mmk (Millipore, USA) were used.

The RNA samples were labelled at 5′-ends with T_4 polynucleotide kinase in the presence of [γ-^{32}P]ATP after dephosphorylation by bacterial alkaline phosphatase [8].

Reagents: RNase A, T_1, T_2 and pronase (Calbiochem, USA); oligo(dT) cellulose (Pharmacia, USA); pyronin (Merck, FRG); T_4 polynucleotide kinase (Boehringer Mannheim, FRG); bacterial alkaline phosphatase (Worthington, USA); [γ-^{32}P]ATP (Isotop, USSR).

3. RESULTS AND DISCUSSION

The duplex (ds) structures isolated from total rat liver high-molecular-mass poly(A)$^+$ RNA by RNase treatment were previously shown to contain three size classes according to electrophoretic mobility [6]. The ds fraction of about 40 bp, as has been demonstrated by comparative fingerprint analysis, is mainly formed by intermolecular interaction of small RNA and poly(A)$^+$ RNA [10].

The fingerprints of the isolated intermolecular duplex structures between total cytoplasmic poly(A)$^+$ RNA and low-molecular-mass RNA are shown in fig.1. The comparison of the oligonucleotide composition of this fraction from control and cortisone-stimulated rat livers (fig.1, 1 and 2) reveals that only a few oligonucleotides (NN 1, 2, 3, 4, 5) are considerably stimulated under the action of the cortisone. Earlier [4] it has been observed that the content of intermolecular duplexes in the population of poly(A)$^+$ RNA increases in response to the hormone. The diversity of the mRNA species induced by cortisone in the cytoplasm [11] is rather high: of a magnitude of 10^3 species (this stimulation is suggested to involve posttranscriptional stages of gene expression) [11]. The diversity of the ds-fraction under consideration is much lower [6]. The fact that not all the ds sequences, but only few of them, are stimulated by the hormone suggests that the different hormone-inducible mRNAs might contain common ds-regions and, consequently, common nucleotide sequences complementary to low-molecular-mass RNAs. We propose that this observation is of functional importance and that these common sequences might serve as structural signals for co-ordinate regulation of posttranscriptional genome expression by the hormone. What part of the mRNA molecule is involved in this interaction remains to be elucidated, but potential base pairing sites might exist in the 3′- and 5′-non-coding sites of mRNA.

The sequencing of the stimulated oligonucleotides (fig.1) is now in progress.

The oligonucleotide map of the small RNA separated by denaturation from the total rat liver cytoplasmic poly(A)$^+$ RNA is shown in figs 2 and 3. The fingerprint reveals the heterogeneity of this fraction. The electrophoresis in 10% PAAG also shows the heterogeneous composition of sacc-RNA (fig.4). The specific character of the fraction of small associated RNA is supported by the observation that different ways of denaturation (heat and formamide) result in the same electrophoretic and fingerprint pattern (not shown). Besides, as can be seen in fig.3, the T_1 fingerprints of the small associated RNA is totally different from the T_1-oligonucleotide map of total small cytoplasmic rat liver RNA.

Under the influence of the hormone, a few species of the sacc-RNA are stimulated (fig.2). This also suggests that the few specific kinds of sacc-RNA are associated with the total diverse population of the glucocorticoid-stimulated messenger RNA in the liver and allows one to propose the possibility of co-ordinate hormonal regulation of posttranscriptional events by small associated RNA. It might also be proposed that the majority of the induced RNA does not contain in-

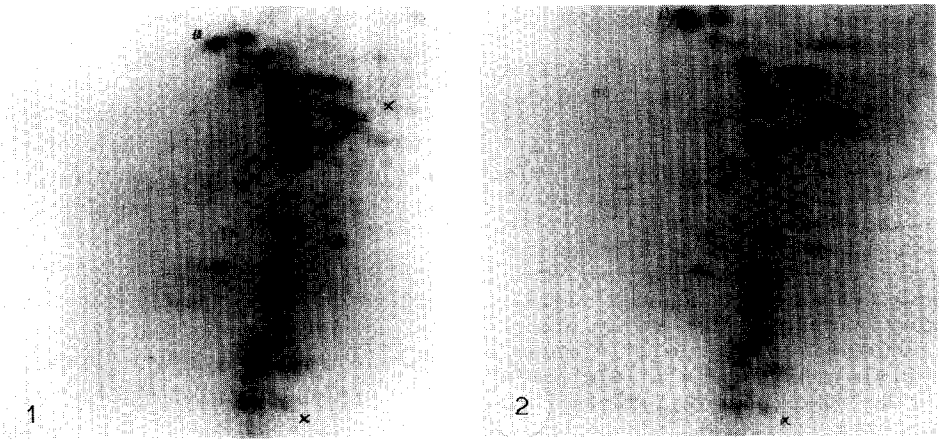

Fig.1. The fingerprints of intermolecular duplexes between high-molecular-mass poly(A)$^+$ RNA and small RNA. (1) Control, (2) cortisone induction, (x) the position of the marker dye.

termolecular duplex regions at all. However, the total content of the intermolecular ds regions of the poly(A)$^+$ RNA population increases under the action of the hormone [6].

A part of the stimulated sequences in sacc-RNA belongs to intermolecular duplexes (figs 1 and 3), the other apparently forms the unpaired ends. The fraction of sacc-RNA may contain the impurities of the destructured during denaturation and purification of high-molecular-mass poly(A)$^+$ RNA, which is reflected by minor spots of oligonucleotides on the fingerprints.

Of special interest is the close similarity of the oligonucleotide composition of small associated cytoplasmic RNA and α-RNA: the component of small nuclear RNP (α). This RNP, as has been shown earlier [12], accepts labelled glucocorticoids ([^{3}H]dexamethasone and [^{3}H]hydrocortisone) in the chromatin of liver nuclei. The fingerprints are presented in fig.3. As can be seen in this figure, several spots are present in different amounts in the two preparations.

The homology of α-RNA and sacc-RNA suggests a dual role for these RNA fractions: par-

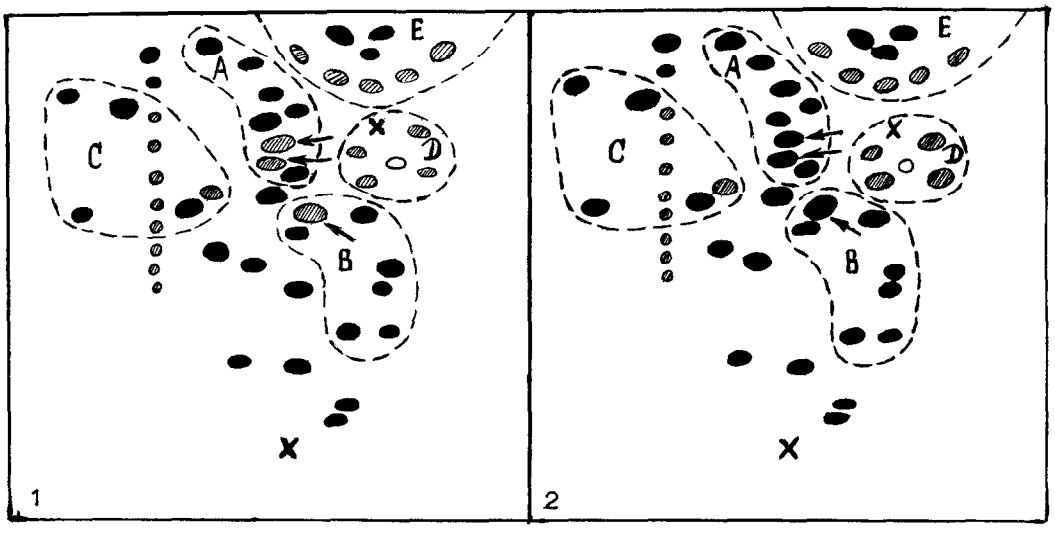

Fig.2. The oligonucleotide maps of sacc-RNA. (1) Control, (2) cortisone-stimulated.

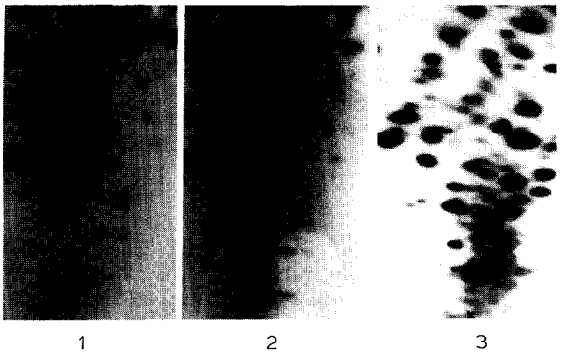

Fig.3. The T_1-fingerprints of small RNA. (1) sacc-RNA, (2) α-RNA, (3) total low-molecular-mass RNA.

ticipation in the changing of mRNA conformation in the cytoplasm and in the regulation of genome expression in chromatin, because α-RNP is tightly bound to it. The suggestions concerning α-RNP function in chromatin have been published earlier [12].

The possible involvement of α-RNA in the formation of mRNA conformation in the cytoplasm is confirmed by the results of blot hybridization of α-RNA with cytoplasmic poly(A)$^+$ RNA (fig.5). As can be seen from this figure, α-RNA, as well as sacc-RNA are capable of interacting complementarily with poly(A)$^+$ RNA. It is possible that in vivo, the whole particle of α-RNP participates in this interaction. The results presented (fig.5, 1 and

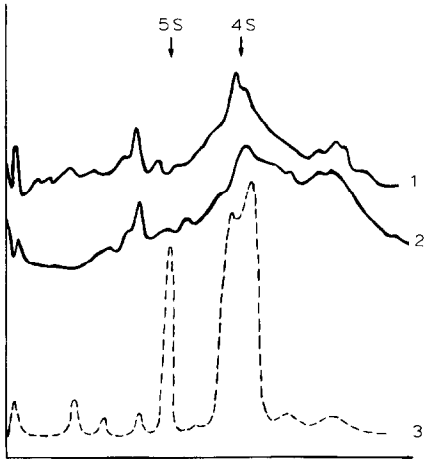

Fig.4. Electrophoresis in 10% PAAG in TBE buffer of small associated RNA (1,2). (1) Control, (2) cortisone-induced, (3) total cytoplasmic low-molecular-mass RNA.

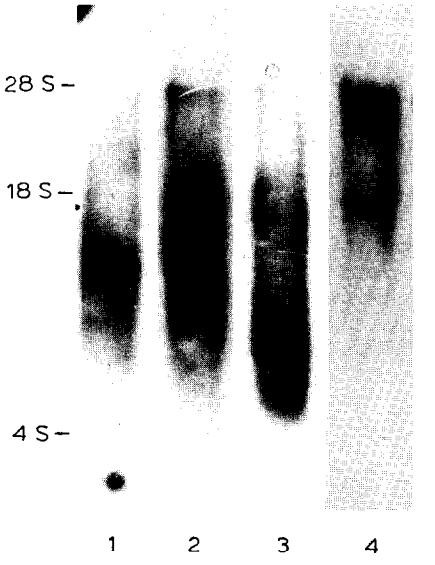

Fig.5. Northern blot hybridization of poly(A)$^+$ RNA (cortisone-stimulated) with: ^{32}P-sacc-RNA from control rats (1), and cortisone-induced animals (2), α-RNA (3). (4) Hybridization of high-molecular-mass poly(A)$^-$ RNA with sacc-RNA.

2) also demonstrate the different patterns of intensity distribution of hybridization zones of sacc-RNA from control and hormone-treated livers with the same poly(A)$^+$ RNA population. This confirms that the specific changes are induced by cortisone in the population of sacc-RNA.

It has become increasingly clear in recent years that the conformation of messenger RNA is important for the regulation of its stability as well as the efficiency of the translation process [13–15]. The results of the present paper suggest that small RNA, associated with poly(A)$^+$ RNA by complementary (antisense) interactions and changing its spatial structure, might participate in the coordinate regulation of gene expression by hormones at the posttranscriptional level. It may be proposed that this regulation may also be of positive character, not only negative as has been shown so far in prokaryotes.

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